ORIGINAL ARTICLE

CQ-397 and CQ-414: antimicrobial activity and spectrum of two fluoroquinolone–cephalosporin, dual-action compounds with carboxamido bonds

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Objective: To evaluate the potential spectrum of activity of two novel dual-action compounds with carboxamido bonds (CQ-397 and CQ-414; Laboratorios Aranda, San Rafael, Mexico) against human pathogens.

Methods: Approximately 800 Gram-positive and Gram-negative aerobic clinical bacteria were tested in vitro using the Mueller-Hinton broth microdilution method of the National Committee of Clinical Laboratory Standards.

Results: CQ-397 (cefamandole+enrofloxacin) and CQ-414 (cefamandole+norfloxacin) were equally potent against Enterobacteriaceae (MIC_{90} range, 0.06–0.5 μg/mL and 0.06–1 μg/mL, respectively). *Citrobacter freundii* (MIC_{90}, 4 μg/mL) and *Providencia* spp. (MIC_{90}, >32 μg/mL) exhibited elevated study drug MICs. Enterobacteriaceae resistant to fluoroquinolones generally remained resistant. CQ-397 and CQ-414 were active against *Stenotrophomonas maltophilia* (MIC_{90}, 4 μg/mL) and oxacillin-susceptible staphylococci (MIC_{90}, 0.25 μg/mL), but not oxacillin-resistant *Staphylococcus aureus* (MIC_{90}, >32 μg/mL), *Staphylococcus epidermidis* (MIC_{90}, 8 μg/mL), and enterococci (MIC_{90}s, 8 to >32 μg/mL). There was no difference in the dual-action drug activity (MIC_{90}, 2 μg/mL) between penicillin-susceptible and -resistant pneumococci. *Haemophilus influenzae* and *Moraxella catarrhalis* were very susceptible (MIC range, ≤0.015–0.06 μg/mL) to both compounds.

Conclusions: The activity of these novel dual-action compounds, formed from the bonding of older antimicrobials, warrants further investigation for potential human and/or animal health use, including toxicology and pharmacokinetics.

Key words: Dual-action compounds, older cephalosporins, enrofloxacin, norfloxacin

INTRODUCTION

CQ-397 and CQ-414 are synthetic broad-spectrum, dual-action combination (DAC) antimicrobials consisting of a cephalosporin (cefamandole-like) linked at the C-7 position by a carboxamido bond to the fluoroquinolones enrofloxacin and norfloxacin, respectively. Antimicrobial drugs having a dual mode of action were first described two decades ago but, unfortunately, toxic side-effects limited their therapeutic use [1]. However, further development of DAC agents led to compounds that combined the cell wall synthesis-inhibitory action of a cephalosporin with the DNA gyrase-inhibiting activity of a quinolone [2]. For example, Ro23-9424 (an ester-linked co-drug of desacetylcefotaxime and fleroxacin) has demonstrated potent activity against Enterobacteriaceae, staphylococci, *Streptococcus* spp., *Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria* spp. and numerous strains resistant to fluoroquinolones or third-generation cephalosporins [3,4]. Another DAC, Ro25-0534 (a combination of a catechol cephalosporin and ciprofloxacin) was generally less potent than Ro23-9424, although it possessed superior activity against *Stenotrophomonas maltophilia* and *Pseudomonas* spp. [5].

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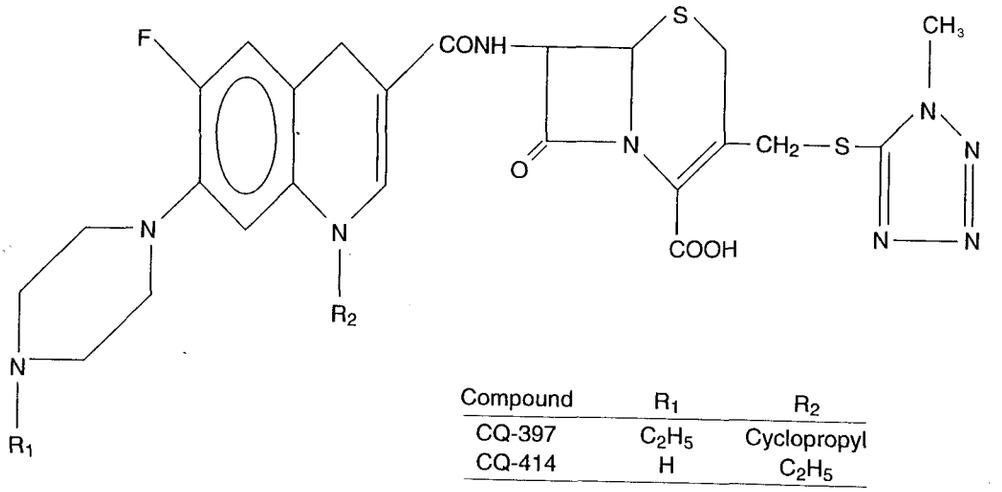

Figure 1 Chemical structures of CQ-397 and CQ-414.

CQ-397 and CQ-414 are structurally similar to the previously studied DAC R023-9424, and have a fluoroquinolone moiety that is linked at its 3′ carboxyl group to the cephalosporin component. However, this structure could limit the candidate DAC to cephalosporin-like activity until the fluoroquinolone is released [2]. Unique to the new Aranda compounds is the carboxyamido bond at the C-7 position, in contrast to the 3′-position bonds utilized in previous DAC drugs. In this study, we evaluated the in vitro potency and spectrum of activity of CQ-397 and CQ-414 (Figure 1) compared with cefamandole, enrofloxacin and norfloxacin. In addition, ceftazidime was added as a third-generation comparator cephalosporin because of its broader and more potent spectrum of activity with respect to cefamandole.

MATERIALS AND METHODS

CQ-397, CQ-414 and enrofloxacin were provided by Laboratories Aranda, S.A. (San Rafael, Mexico). Cefamandole and ceftazidime were obtained from Eli Lilly and Co. (Indianapolis, IN), and norfloxacin from Merck, Inc. (Rahway, NJ).

Nearly 800 Gram-positive and Gram-negative aerobic bacteria were tested (Tables 1–3). The organisms were recent clinical isolates (1993–95) cultured from blood and other sterile body fluids (not including urine) in patients at the University of Iowa Hospitals and Clinics (Iowa City, IA). The strains were stored at −70°C and subcultured twice prior to being tested.

Minimum inhibitory concentrations (MICs) were determined in cation-adjusted Mueller-Hinton broth microdilution trays (Prepared Media Microbiologics, Tualatin, OR). Fastidious organisms, such as *Streptococcus* spp., *Corynebacterium jeikeium*, *Bacillus cereus* and *Haemophilus influenzae*, were grown in medium supplemented with 5% lysed horse blood. After overnight incubation, the trays were examined for growth and MICs were determined according to National Committee for Clinical Laboratory Standards (NCCLS) methods [6,7]. Interpretive breakpoint criteria for norfloxacin, cefamandole and ceftazidime followed NCCLS [6] guidelines, while enrofloxacin and the new DACs were assigned the susceptible breakpoints of ≤1 μg/mL and ≤8 μg/mL, respectively.

RESULTS AND DISCUSSION

Table 1 lists the results of the study compounds tested against 250 strains of Enterobacteriaceae. CQ-397 and CQ-414 were equally active (MIC_{90} range, 0.06–0.5 μg/mL and 0.06–1 μg/mL, respectively) against this family of bacteria. *Citrobacter freundii* (MIC_{90}, 4 μg/mL) and the *Providencia* spp. (MIC_{90}, >32 μg/mL) demonstrated greater resistance to the DAC drugs; however, the comparison cephalosporins, cefamandole and ceftazidime, remained active against the *Providencia* spp. strains (MIC_{90} range, 0.25–4 μg/mL). Several species (*Enterobacter aerogenes, Enterobacter cloacae, Klebsiella oxytoca, Morganella morganii, Panteoa agglomerans, Proteus vulgaris* and *Serratia marcescens*) that exhibited resistant-range MIC results (MIC_{90}, >32 μg/mL) to cefamandole were more susceptible to both DAC drugs (MIC_{90} range, 0.25–1 μg/mL). In general, when the

Table 1 CQ-397 and CQ-414 antimicrobial activity and spectrum tested against 250 Enterobacteriaceae clinical isolates by NCCLS dilution methods

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Gram-negative bacilli					
Citrobacter freundii (20)	CQ-397	0.25	4	≤0.015 to >32	90 (85)
	CQ-414	0.12	8	≤0.015 to >32	90 (85)
	Enrofloxacin	0.03	2	≤0.008 to >16	90
	Norfloxacin	0.12	2	0.015 to >16	95
	Cefamandole	>32	>32	0.5 to >32	35
	Ceftazidime	>16	>32	0.12 to >16	45
Citrobacter koseri (10)	CQ-397	0.03	0.06	0.03–0.25	100 (100)
	CQ-414	0.03	0.06	0.03–0.25	100 (100)
	Enrofloxacin	≤0.008	0.015	≤0.008–0.06	100
	Norfloxacin	0.03	0.06	0.03–0.25	100
	Cefamandole	0.5	2	0.25–4	100
	Ceftazidime	0.12	0.5	0.06–1	100
Enterobacter aerogenes (20)	CQ-397	0.12	0.5	0.03 to >32	95 (90)
	CQ-414	0.06	0.5	0.03 to >32	95 (90)
	Enrofloxacin	0.03	0.25	0.015 to >16	95
	Norfloxacin	0.06	0.5	0.06 to >16	90
	Cefamandole	8	>32	0.5 to >32	50
	Ceftazidime	0.25	>16	0.06 to >16	55
Enterobacter cloacae (20)	CQ-397	0.06	0.5	≤0.015 to >32	90 (90)
	CQ-414	0.06	0.5	0.03 to >32	90 (90)
	Enrofloxacin	0.015	0.12	≤0.008 to >16	90
	Norfloxacin[a]	0.06	0.25	0.03 to >16	90
	Cefamandole	4	>32	1 to >32	65
	Ceftazidime	0.25	>16	0.12 to >16	80
Escherichia coli (20)	CQ-397	0.03	0.06	0.03–32	90 (90)
	CQ-414	0.03	0.06	0.03–32	90 (90)
	Enrofloxacin	0.015	0.015	≤0.008–16	90
	Norfloxacin	0.06	0.12	0.03 to >16	90
	Cefamandole	1	8	0.25 to >32	90
	Ceftazidime	0.12	1	≤0.03 to >16	95
Klebsiella oxytoca (10)	CQ-397	0.06	0.12	0.06–0.25	100 (100)
	CQ-414	0.06	0.12	0.06–0.25	100 (100)
	Enrofloxacin	0.03	0.03	0.015–0.12	100
	Norfloxacin	0.12	0.25	0.06–0.5	100
	Cefamandole	4	>32	0.5 to >32	70
	Ceftazidime	0.25	1	0.06–2	100
Klebsiella pneumoniae (20)	CQ-397	0.12	0.5	0.03 to >32	90 (90)
	CQ-414	0.06	0.05	0.03 to >32	90 (90)
	Enrofloxacin	0.03	0.12	0.015 to >16	90
	Norfloxacin	0.12	0.5	0.06 to >16	90
	Cefamandole	1	4	0.25 to >32	90
	Ceftazidime	0.12	1	≤0.03–16	95
Morganella morganii (10)	CQ-397	0.12	0.25	0.06–0.25	100 (100)
	CQ-414	0.12	0.25	0.06–0.25	100 (100)
	Enrofloxacin	0.03	0.06	0.015–0.12	100
	Norfloxacin	0.03	0.06	0.015–0.06	100
	Cefamandole	16	>32	0.5 to >32	30
	Ceftazidime	0.25	8	≤0.03 to >16	90

Table 1—*continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Gram-negative bacilli					
Pantoea agglomerans (10)	CQ-397	0.06	0.06	≤0.015–0.06	100 (100)
	CQ-414	0.03	0.06	≤0.015–0.06	100 (100)
	Enrofloxacin	0.015	0.015	≤0.008–0.03	100
	Norfloxacin	0.06	0.12	0.03–0.25	100
	Cefamandole	2	>32	0.25 to >32	70
	Ceftazidime	0.12	>16	0.12 to >32	70
Proteus mirabilis (20)	CQ-397	0.25	0.25	0.12–0.5	100 (100)
	CQ-414	0.25	0.25	0.12–0.5	100 (100)
	Enrofloxacin	0.06	0.12	0.03–0.12	100
	Norfloxacin	0.06	0.06	0.03–0.25	100
	Cefamandole	0.5	1	0.25 to >32	100
	Ceftazidime	≤0.03	≤0.03	≤0.03–0.06	100
Proteus vulgaris (10)	CQ-397	0.12	0.25	0.12–1	100 (100)
	CQ-414	0.12	0.25	0.12–1	100 (100)
	Enrofloxacin	0.03	0.06	0.03–0.5	100
	Norfloxacin	0.03	0.06	0.03–0.06	100
	Cefamandole	>32	>32	8 to >32	100
	Ceftazidime	0.06	0.06	≤0.03–0.06	100
Providencia rettgeri (10)	CQ-397	0.25	>32	0.12 to >32	80 (90)
	CQ-414	0.25	32	0.12 to >32	80 (80)
	Enrofloxacin	0.06	8	0.03 to >16	80
	Norfloxacin	0.06	8	0.03 to >16	80
	Cefamandole	≤0.06	0.5	≤0.06 to >32	90
	Ceftazidime	≤0.03	0.5	≤0.03–0.5	100
Providencia stuartii (10)	CQ-397	0.5	32	0.06 to >32	80 (70)
	CQ-414	0.5	>32	0.06 to >32	80 (70)
	Enrofloxacin	0.25	8	0.03 to >16	60
	Norfloxacin	0.25	8	0.12 to >16	70
	Cefamandole	2	4	0.5–8	100
	Ceftazidime	0.12	0.5	0.03–0.5	100
Salmonella enteritidis (10)	CQ-397	0.06	0.12	0.06–0.12	100 (100)
	CQ-414	0.06	0.06	0.06–0.12	100 (100)
	Enrofloxacin	0.03	0.03	0.015–0.03	100
	Norfloxacin	0.06	0.06	0.03–0.12	100
	Cefamandole	0.25	1	0.12–2	100
	Ceftazidime	0.12	0.25	0.12–0.25	100
Serratia marcescens (20)	CQ-397	0.5	0.5	0.25 to >32	80 (80)
	CQ-414	0.5	1	0.25 to >32	80 (80)
	Enrofloxacin	0.12	0.25	0.06–16	80
	Norfloxacin	0.25	0.5	0.12 to >16	80
	Cefamandole	>32	>32	4 to >32	15
	Ceftazidime	0.25	1	0.12–1	100
Shigella spp. (10)	CQ-397	0.03	0.06	≤0.015–0.06	100 (100)
	CQ-414	0.03	0.03	≤0.015-0.03	100 (100)
	Enrofloxacin	≤0.008	0.015	≤0.008–0.015	100
	Norfloxacin	0.03	0.06	0.03–0.06	100
	Cefamandole	0.5	2	0.25–2	100
	Ceftazidime	0.06	0.12	0.06–0.12	100

Table 1—*continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Gram-negative bacilli					
Yersinia enterocolitica (10)	CQ-397	0.03	0.06	0.03–0.12	100 (100)
	CQ-414	0.03	0.12	0.03–0.12	100 (100)
	Enrofloxacin	0.015	0.06	≤0.008–0.06	100
	Norfloxacin	0.06	0.06	0.03–0.12	100
	Cefamandole	4	4	2–8	100
	Ceftazidime	0.25	1	0.06–4	100
Other Enterobacteriaceae (10)[b]	CQ-397	0.06	0.12	0.03–0.5	100 (100)
	CQ-414	0.06	0.12	0.03–0.5	100 (100)
	Enrofloxacin	0.015	0.03	≤0.008–0.25	100
	Norfloxacin	0.03	0.06	0.015–1	100
	Cefamandole	8	>32	0.25 to >32	50
	Ceftazidime	0.12	>16	0.06 to >16	80

[a]Breakpoint criteria per National Committee for Clinical Laboratory Standards [6] for norfloxacin, cefamandole and ceftazidime. The enrofloxacin-susceptible breakpoint was assigned at ≤1 μg/mL (same as ciprofloxacin) and the commonly used cephalosporin breakpoint of ≤8 μg/mL was applied to CQ-397 and CQ-414. The % susceptible values parentheses indicate the ≤2 μg/mL breakpoint.
[b]Includes six species: *Enterobacter sakazakii* (two strains), *Enterobacter taylorae* (two strains), *Hafnia alvei* (one strain), *Klebsiella ozaenae* (one strain), *Salmonella typhi* (two strains) and *Serratia liquefaciens* (two strains).

Table 2 CQ-397 and CQ-414 antimicrobial activity and spectrum tested against various non-enteric Gram-negative clinical isolates by NCCLS dilution methods

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Gram-negative bacilli					
Acinetobacter spp. (10)	CQ-397	0.06	0.12	0.03–0.4	100 (100)
	CQ-414	0.06	0.12	0.03–0.5	100 (100)
	Enrofloxacin	0.03	0.06	0.015–0.12	100
	Norfloxacin	2	8	1–16	90
	Cefamandole	>32	>32	32 to >32	0
	Ceftazidime	2	8	1–8	100
Pseudomonas aeruginosa (30)	CQ-397	1	16	0.5–32	77 (67)
	CQ-414	1	16	0.5–32	77 (67)
	Enrofloxacin	0.5	4	0.25–8	73
	Norfloxacin	0.5	2	0.25–4	100
	Cefamandole	>32	>32	>32	0
	Ceftazidime	2	8	0.5 to >16	0
Stenotrophomonas maltophilia (10)	CQ-397	1	4	0.25–1	100 (100)
	CQ-414	1	4	0.25–1	100 (100)
	Enrofloxacin	0.25	2	0.12–1	100
	Norfloxacin	8	>16	4 to >16	10
	Cefamandole	>32	>32	16 to >32	0
	Ceftazidime	1	8	1–16	90
Moraxella catarrhalis β-lactamase-positive BRO-I (10)	CQ-397	0.03	0.06	0.03–0.06	100 (100)
	CQ-414	0.03	0.06	0.03–0.06	100 (100)
	Enrofloxacin	0.015	0.015	0.008–0.015	100
	Norfloxacin	0.12	0.12	0.06–0.12	100
	Cefamandole	2	4	2–4	100
	Ceftazidime	≤0.03	≤0.03	≤0.03	100

Table 2—*continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Gram-negative bacilli					
Moraxella catarrhalis					
β-lactamase-positive					
BRO-2 (10)	CQ-397	0.06	0.06	0.03–0.06	100 (100)
	CQ-414	0.06	0.06	0.03–0.06	100 (100)
	Enrofloxacin	0.015	0.015	≤0.008–0.015	100
	Norfloxacin	0.12	0.12	0.06–0.12	100
	Cefamandole	2	4	2–4	100
	Ceftazidime	≤0.03	≤0.03	≤0.03	100
β-lactamase-negative (10)	CQ-397	0.06	0.06	0.03–0.06	100 (100)
	CQ-414	0.03	0.06	0.03–0.06	100 (100)
	Enrofloxacin	0.015	0.015	0.015	100
	Norfloxacin	0.12	0.12	0.06–0.12	100
	Cefamandole	0.12	0.5	0.12–0.5	100
	Ceftazidime	≤0.03	≤0.03	≤0.03–0.12	100
Haemophilus influenzae					
β-lactamase-positive (20)	CQ-397	≤0.015	0.03	≤0.015–0.06	100 (100)
	CQ-414	0.06	0.06	0.06	100 (100)
	Enrofloxacin	0.015	0.015	0.015–0.03	100
	Norfloxacin	0.03	0.06	0.03–0.06	100
	Cefamandole	1	4	0.5–4	100
	Ceftazidime	≤0.03	0.06	≤0.03–0.06	100
β-lactamase-negative,	CQ-397	≤0.015	≤0.015	≤0.015–0.03	100 (100)
Ampicillin-susceptible (20)	CQ-414	0.06	0.06	0.03–0.06	100 (100)
	Enrofloxacin	0.015	0.015	0.015	100
	Norfloxacin	0.03	0.03	0.015–0.06	100
	Cefamandole	0.25	0.5	≤0.06–1	100
	Ceftazidime	0.06	0.06	≤0.03–0.12	100
Ampicillin-resistant (10)	CQ-397	0.03	0.03	≤0.015–0.03	100 (100)
	CQ-414	0.06	0.06	≤0.015–0.06	100 (100)
	Enrofloxacin	0.015	0.015	0.008–0.015	100
	Norfloxacin	0.06	0.06	0.03–0.06	100
	Cefamandole	32	>32	8 to >32	0
	Ceftazidime	1	4	0.12 to >16	70

[a]Breakpoint criteria per NCCLS [6] for norfloxacin, cefamandole and ceftazidime. The enrofloxacin susceptible breakpoint was assigned at ≤1 μg/mL (same as ciprofloxacin) and the commonly used cephalosporin breakpoint of ≤8 μg/mL was applied to CQ-397 and CQ-414. If a fluoroquinolone breakpoint (≤2 μg/mL) was used for CQ-397 or CQ-414, the spectrum is indicated in parentheses.

activities of CQ-397 and CQ-414 are compared with those of their component fluoroquinolones (enrofloxacin and norfloxacin), the best component drug was two- to four-fold more active than CQ-397 and CQ-414. The spectrum and potency of the 'third-generation' cephalosporin comparator were superior to those of both DAC drugs.

Table 2 contains the activity results for the study drugs tested against non-enteric Gram-negative organisms. The species causing respiratory tract infection, *Haemophilus influenzae* and *Moraxella catarrhalis*, were usually susceptible to all tested antimicrobial agents. Cefamandole had slightly elevated MICs for the β-lactamase-negative ampicillin-resistant (BLNAR) *Haemophilus influenzae* strains, but none of the remaining compounds was significantly affected by strains possessing enzymatic or penicillin-binding-protein-mediated resistances. Norfloxacin (MIC_{90}, 2 μg/mL) was the most active compound against *Pseudomonas aeruginosa*. The DACs (MIC_{90} range, 0.12–4 μg/mL) were more potent than the other core structure compound cefamandole (MIC_{90}, >32 μg/mL) against *Acinetobacter* spp. and *Stenotrophomonas maltophilia*.

Table 3 CQ-397 and CQ-414 antimicrobial activity and spectrum tested against Gram-positive clinical isolates by NCCLS dilution methods

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Staphylococcus aureus					
Oxacillin-susceptible (100)	CQ-397	0.25	0.25	0.06–32	98 (98)
	CQ-414	0.25	0.25	0.06–32	98 (98)
	Enrofloxacin	0.06	0.12	≤0.08–2	98
	Norfloxacin	1	2	0.12 to >16	96
	Cefamandole	0.5	0.5	≤0.06–1	100
	Ceftazidime	4	8	4–16	98
Oxacillin-resistant (50)	CQ-397	32	>32	0.12 to >32	36 (34)
	CQ-414	32	>32	0.12 to >32	38 (34)
	Enrofloxacin	4	>16	0.03 to >16	34
	Norfloxacin	>16	>16	0.5 to >16	26
	Cefamandole	8	16	1–32	62
	Ceftazidime	>16	>16	8 to >16	2
Staphylococcus epidermidis					
Oxacillin-susceptible (23)	CQ-397	0.25	0.25	0.12–0.25	100 (100)
	CQ-414	0.25	0.25	0.06–0.25	100 (100)
	Enrofloxacin	0.06	0.12	0.03–0.12	100
	Norfloxacin	0.5	1	0.25–1	100
	Cefamandole	0.12	0.25	0.12–1	100
	Ceftazidime	4	4	4–16	96
Oxacillin-resistant (27)	CQ-397	0.25	8	0.12 to >32	96 (89)
	CQ-414	0.25	8	0.12 to >32	93 (89)
	Enrofloxacin	0.06	1	0.03–4	93
	Norfloxacin	0.5	16	0.25 to >16	78
	Cefamandole	2	4	0.5–8	100
	Ceftazidime	>16	>16	8 to >16	15
Staphylococcus haemolyticus					
Oxacillin-susceptible (20)	CQ-397	0.12	0.12	0.12–0.25	100 (100)
	CQ-414	0.12	0.12	0.06–0.25	100 (100)
	Enrofloxacin	0.03	0.03	0.015–0.06	100
	Norfloxacin	0.25	0.5	0.03–0.5	100
	Cefamandole	0.25	0.5	0.12–0.5	100
	Ceftazidime	8	8	4–16	86
Oxacillin-resistant (13)	CQ-397	>32	>32	0.12 to >32	8 (0)
	CQ-414	>32	>32	0.12 to >32	8 (0)
	Enrofloxacin	>16	>16	0.03 to >16	0
	Norfloxacin	>16	>16	0.25 to >16	0
	Cefamandole	>32	>32	2 to >32	30
	Ceftazidime	>16	>16	>16	0
Coagulase-negative staphylococci (20)	CQ-397	0.25	0.5	0.06 to >32	100 (95)
	CQ-414	0.25	0.5	0.06 to >32	100 (95)
	Enrofloxacin	0.06	0.12	0.015 to >16	95
	Norfloxacin	0.5	1	0.06 to >16	95
	Cefamandole	0.25	2	0.12–2	100
	Ceftazidime	8	>16	2 to >16	60
Streptococcus					
Group A (20)	CQ-397	1	1	0.25–4	100 (95)
	CQ-414	1	1	0.25–4	100 (95)
	Enrofloxacin	0.5	0.5	0.12–2	95
	Norfloxacin	2	4	1 to >16	95
	Cefamandole	≤0.06	≤0.06	≤0.06	100
	Ceftazidime	0.06	0.25	0.06–0.5	100

Table 3—*continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Streptococcus					
Group B (20)	CQ-397	1	2	1–2	100 (100)
	CQ-414	1	2	1–2	100 (100)
	Enrofloxacin	0.5	1	0.5–1	100
	Norfloxacin	4	8	2–8	85
	Cefamandole	≤0.06	≤0.06	≤0.06	100
	Ceftazidime	0.5	0.5	0.25–0.5	100
Group C (10)	CQ-397	1	1	0.25–2	100 (100)
	CQ-414	1	1	0.25–2	100 (100)
	Enrofloxacin	0.5	0.5	0.12–1	100
	Norfloxacin	2	5	0.5–4	100
	Cefamandole	≤0.06	0.25	≤0.06–4	100
	Ceftazidime	0.5	2	0.12–4	100
Group G (10)	CQ-397	0.5	1	0.5–1	100 (100)
	CQ-414	0.5	1	0.5–1	100 (100)
	Enrofloxacin	0.25	0.5	0.25–0.5	100
	Norfloxacin	2	2	1–4	100
	Cefamandole	≤0.06	≤0.06	≤0.06–0.12	100
	Ceftazidime	0.25	2	0.12–2	100
Streptococcus pneumoniae					
Penicillin-susceptible (17)	CQ-397	2	2	0.06–4	100 (94)
	CQ-414	1	2	0.06–4	100 (94)
	Enrofloxacin	1	1	0.015–2	94
	Norfloxacin	4	8	0.06–8	76
	Cefamandole	0.06	0.25	0.06–0.25	100
	Ceftazidime	0.25	0.25	0.06–2	100
Penicillin-intermediate and -resistant (13)	CQ-397	1	2	0.5–2	100 (100)
	CQ-414	1	2	0.5–2	100 (100)
	Enrofloxacin	0.5	1	0.25–1	100
	Norfloxacin	8	8	4–8	100
	Cefamandole	2	8	0.25–32	92
	Ceftazidime	8	16	0.5 to >16	77
Enterococcus spp.					
Vancomycin-susceptible (25)	CQ-397	4	>32	1 to >32	56 (44)
	CQ-414	4	>32	1 to >32	56 (44)
	Enrofloxacin	1	>16	1 to >16	52
	Norfloxacin	4	>16	2 to >16	56
	Cefamandole	>32	>32	16 to >32	0
	Ceftazidime	>16	>16	4 to >16	4
Vancomycin-resistant *vanA* (9)	CQ-397	32	>32	1 to >32	11 (11)
	CQ-414	32	>32	1 to >32	11 (11)
	Enrofloxacin	>16	>16	4 to >16	11
	Norfloxacin	>16	>16	2 to >16	11
	Cefamandole	>32	>32	32 to >32	0
	Ceftazidime	>16	>16	>16	0
vanB (10)	CQ-397	>32	>32	0.5 to >32	10 (10)
	CQ-414	>32	>32	0.5 to >32	10 (10)
	Enrofloxacin	>16	>16	0.25 to >16	10
	Norfloxacin	>16	>16	8 to >16	10
	Cefamandole	>32	>32	>32	0
	Ceftazidime	>16	>16	>16	0

Table 3—*continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible[a]
Enterococcus spp.					
Vancomycin-resistant					
vanC (15)	CQ-397	2	8	1–8	100 (73)
	CQ-414	2	8	1–8	100 (60)
	Enrofloxacin	1	2	0.5–2	75
	Norfloxacin	8	8	4–8	27
	Cefamandole	16	32	2 to >32	47
	Ceftazidime	>16	>16	>16	0
Corynebacterum jeikeium (10)	CQ-397	32	>32	0.25 to >32	20 (10)
	CQ-414	32	>32	0.25 to >32	20 (10)
	Enrofloxacin	16	16	0.12 to >16	10
	Norfloxacin[a]	>16	>16	1 to >16	10
	Cefamandole	>32	>32	0.5 to >32	10
	Ceftazidime	>16	>16	4 to >16	10
Bacillus cereus (7)	CQ-397	0.06	–	0.06–0.12	100 (100)
	CQ-414	0.06	–	0.06–0.12	100 (100)
	Enrofloxacin	0.03	–	0.03–0.06	100
	Norfloxacin	0.25	–	0.25	100
	Cefamandole	16	–	8–32	10
	Ceftazidime	>16	–	>16	0

[a]Breakpoint criteria per NCCLS [6] for norfloxacin, cefamandole and ceftazidime. The enrofloxacin-susceptible breakpoint was assigned at ≤1 μg/mL (same as ciprofloxacin) and the commonly used cephalosporin breakpoint of ≤8 μg/mL was applied to CQ-397 and CQ-414. If a fluoroquinolone breakpoint (≤2 μg/mL) was used for CQ-397 or CQ-414, the spectrum is indicated in parentheses.

The activities of DAC drugs and the comparison compounds tested against Gram-positive organisms are listed in Table 3. Oxacillin-susceptible staphylococci were susceptible to CQ-397 and CQ-414 (MIC_{90} range, 0.12–0.5 μg/mL); however, oxacillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus* strains had very elevated MIC results (256- and 128-fold, respectively). Although oxacillin-resistant *Staphylococcus epidermidis* MICs were generally 16-fold greater than those of oxacillin-susceptible isolates, the vast majority (90%) still had DAC MICs of ≤8 μg/mL. CQ-397 was less active (MIC_{90}, 8 μg/mL) than the component drugs enrofloxacin (MIC_{90}, 1 μg/mL) and cefamandole (MIC_{90}, 4 μg/mL) against oxacillin-resistant *Staphylococcus epidermidis*, indicating potential 'antagonism'. Only cefamandole had any measurable activity against oxacillin-resistant *Staphylococcus haemolyticus*. The remaining coagulase-negative *Staphylococcus* spp. were less susceptible to ceftazidime (40% resistant) than to the remaining studied antimicrobials.

There was no difference in the CQ-397 and CQ-414 potencies (MIC_{90}, 2 μg/mL) between penicillin-susceptible and -resistant *Streptococcus pneumoniae* strains. CQ-397 had a slightly higher MIC (two- to 16-fold) than the component drugs against some β-haemolytic streptococci, again suggesting possible 'antagonism'. DAC potency against *Enterococcus* spp. (100% susceptible at ≤8 μg/mL) carrying the *vanC* gene was greater than that against vancomycin-susceptible strains (56% susceptible at ≤8 μg/mL). Both DACs were more active (MIC_{90}, 0.12 μg/mL) than cefamandole (MIC_{90}, 32 μg/mL) and ceftazidime (MIC_{90}, >16 μg/mL) against *Bacillus cereus*. All of the study compounds (MIC_{90}, ≥16 μg/mL) were inactive against *Corynebacterium jeikeium*.

DAC antimicrobials continue to be promising agents with potential clinical use in humans and/or animals [1]. A wide variety of candidate cephem–fluoroquinolone combinations have been studied using various linking bonds. The use of older fused agents (norfloxacin+a second-generation cephalosporin) indicates that activity can be enhanced by this process and applications to human practice could include DACs with known safety and efficacy (component drug history). We anxiously await the results of expanded in vitro trials of CQ-397 and CQ-414, or similar compounds.

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